EFFECT OF pH AND IONIC STRENGTH ON PERMEATE FLUX DURING SEPARATION OF *Lactobacillus plantarum* BY USING HOLLOW FIBER CROSSFLOW MICROFILTRATION

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ABSTRACT

Microfiltration is a separation process used for cell harvesting in downstream process. Current research focused on the factor affecting the process of cell separation from fermentation broth to be recycled into the fermenter. The efficiency of solute separation by microfiltration can be influenced by solution pH and ionic strength. The objectives of this research is to study the effects of pH solution which is pH 4.5, 5.5, 6.5, 7.5, 8.5 and ionic strength on permeate flux of separation of *Lactobacillus plantarum* bacteria. For this research, the 0.2, 0.4, 0.6, 0.8 and 1.0 M of salt concentration are also used. At pH 8.5, permeate flux is the highest due to the electrostatic repulsion between the *Lactobacillus plantarum* bacteria and the surface of the membrane. The lowest permeate flux is at 1.0 M of ionic strength due to compaction of membrane and results in reduction of effective permeability. As conclusion, flux can be affected by pH solution and addition of salt. Increase in pH solution resulted in increase in permeate flux and addition of salt decreases permeate flux.

ABSTRAK

Penapis mikro adalah satu proses pemisahan yang digunakan di dalam process pemisahan hiliran. Kajian terkini lebih mengfokuskan kepada faktor yang mempengaruhi proses pemisahan sel daripada campuran penapaian untuk dikitar semula ke dalam penapai. Kecekapan pemisahan bahan larut oleh penapis mikro dipengaruhi oleh pH dan kekuatan ionik. Tujuan kajian ini adalah untuk mengkaji kesan pH larutan iaitu pH 4.5, 5.5, 6.5, 7.5, 8.5 dan kekuatan ionik kepada arus resapan pemisahan bacteria *Lactobacillus plantarum* . Dalam kajian ini, 0.2, 0.4, 0.6, 0.8 dan 1.0 molar kepekatan garam juga digunakan. Pada pH 8.5, arus resapan pemisahan *Lactobacillus plantarum* adalah paling tinggi disebabkan penolakan elektrostatik antara bacteria *Lactobacillus plantarum* dan permukaan penapis. Pada 1.0 molar kekuatan ionik, arus resapan adalah paling rendah disebabkan kepadatan penapis dan menyebabkan pengurangan resapan efektif. Sebagai kesimpulan, arus resapan boleh dipengaruhi oleh pH larutan dan penambahan garam. Arus resapan lebih tinggi apabila pH larutan bertambah dan penambahan garam mengurangkan arus resapan.

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LIST OF SYMBOLS

µm	-	Micrometer
Psig	-	Pound-force per square inch gauge (pressure)
°C	-	Degree celcius
Ml	-	Milliliter
Rpm	-	Revolutions per minute
M	-	Molarity
J	-	Permeate flux
A	-	Area
Cm^3	-	Centimeter cube
NaOH	-	Sodium hydroxide
HCl	-	Hydrocloric Acic
n	-	Moles
KH_2PO_4	-	Potassium dihydrogen phosphate
$KHC_8H_4O_4$	-	Potassium hydrogen phatalate

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fermentation broths are complex aqueous mixtures of cells, soluble extracellular products, intracellular products and converted substrate or unconvertible components of a process called fermentation. As with other chemical process, fermentation for producing products is also aimed at minimizing production costs. Besides minimizing the cost, in order to improve fermentation efficiency and production rate, reusing cells hold promises (Hoek, 2003).

Production of sorbitol is one of the fermentation processes which using *Lactobacillus plantarum* in order to convert the glucose and produces the sugar alcohol, sorbitol. In context of sorbitol production, the *Lactobacillus plantarum* bacteria possess some relevant characteristics. It is a food grade microorganism belonging to the group of lactic acid bacteria and largely found as the dominant species in the last step of natural food raw material fermentation. There are a few microorganism have been suggested as potential sorbitol producers, but *Lactobacillus plantarum* bacteria is the best choose in order to achieve high level sorbitol production by fermentation (Ladero *et al.*, 2007).

Sorbitol is also referred as D-glucitol, is naturally found in many fruits, such as berries, cherries and apple. The worldwide production of sorbitol is estimated to be larger than 500000 tonnes per year and the market is continuously increasing. This polyol has a relative sweetness of around sixty percent compared to sucrose.

Based on these properties, sorbitol is widely used in a range of food products such as confectionery, chewing gums, candy, desserts, ice cream, diabetic foods as sweetener, humectants, texturizer and softener. In addition, sorbitol is the starting material for the production of pharmaceutical compounds such as sorbose and ascorbic acid (Ladero *et al.*, 2007).

In order to minimize the production costs, improve efficiency and production rate, usage of membrane separation to separate the bacteria cell is the best way to achieve the goals because membrane nowadays have gained wide acceptance and made significant inroads against competing technologies in many areas because of flexibility and performance reliability, cost competitiveness and environmental awareness. Besides that, the advantages of using membrane including good process ability, inexpensive production and low operating cost. In short, it offers low capital cost, low energy consumption, ease of operation and cost effectiveness (Sarif, 2005). There are four types of membrane process. They are microfiltration, ultrafiltration, nanofiltration and reverse osmosis (Ghosh, 2006).

1.2 Problem Statement

The percent of cell retention from fermentation broth that can be recycled back to the bioreactor may be affected by the pH of the fermentation broth used because the efficiency of membrane is influenced by pH (Ghosh, 2003). The percent might be too low or zero to be recycled if the pH can cause the pore size of membrane bigger because of the permeation of the cell through the membrane. The membrane morphology may be affected by the pH of the fermentation broth because Rubia states that pH can have significant effect on both fouling and rejection because of the changing of pores size of membrane. The membrane pore size can decrease and increase due to the changing of pH. If the pH of the fermentation broth causes the membrane pores size bigger, the bacteria in the fermentation broth can pass through the membrane, the product may be contaminated and the bacteria cell cannot be recycled back to be used for other fermentation process will cause wastes of money.

Besides that, it can also causes the wasting of time to culture the bacteria for some days before the fermentation process, compared with recycling the bacteria by harvesting from fermentation broth by using microfiltration process. It will also cause the waste of money when the membrane should be replaced so many times because of the fouling, affected by the pH of the fermentation broth.

Membrane fouling is one of the critical phenomena governing the performance of microfiltration separation because fouling causes flux decline. pH and ionic strength are some of factors that can affect the membrane separation (Ghosh, 2003). The different pH and ionic strength causes the different in permeate flux. Hence, the fouling can causes money and time consuming. Because of that, remedies should be done to increase the flux and avoid fouling.

1.3 Objectives

1. To study the effect of pH and ionic strength on membrane flux during separation of *Lactobacillus plantarum* bacteria.

2. To study the effects of fermentation broth pH on permeate flux of separation of *Lactobacillus plantarum* bacteria.

1.4 Scope of Study

In order to achieve the objectives, the following scopes have been identified.

1. Study of the culture process of *Lactobacillus plantarum* bacteria

2. The study of separation of *Lactobacillus plantarum* separation by using hollow fiber cross flow microfiltration

3. The study of pH and ionic strength effect on permeate flux during separation of *Lactobacillus plantarum* bacteria. The range of pH that is used is between 4.5 until 8.5. They are 4.5, 5.5, 6.5, 7.5 and 8.5. For ionic strength, the range which is used between 0.2 M until 1.0 M of ionic strength. They are 0.2, 0.4, 0.6, 0.8 and 1.0 (Yun, J. 1999)

1.4 Rationale and Significance

Microfiltration is a separation process used for cell harvesting in downstream process. Current research focused on the factor affecting the process of cell separation from fermentation broth in order to identify the amount of bacteria cell that can be recycled back into fermentation tank (Kaghazchi *et al.*, 2000). The study of fermentation broth pH effects on the *Lactobacillus plantarum* is to determine whether microfiltration membrane separation is suitable for the separation of *Lactobacillus plantarum* and to determine the effects of the fermentation pH on the permeate flux during *Lactobacillus plantarum* separation.

pH and ionic strength are two of factors that can affect the membrane separation (Ghosh, 2003). The remedies is one of the way to enhance the flux and increase the profits because the study of pH and ionic strength effects on *Lactobacillus plantarum* separation can help in determination of the optimum pH and ionic strength that used be used for separation process of *Lactobacillus plantarum*

from the fermentation broth in order to achieve high permeate flux and one hundred percent of *Lactobacillus plantarum* bacteria retention. Besides that, it can avoid fouling of the membrane, money and time consuming.

CHAPTER 2

LITERATURE REVIEW

2.1 Sorbitol

Sorbitol also referred as glucitol, $C_6H_{14}0_6$ as shown in figure 2.1 (Chun *et al*., 1988) classified as sugar alcohols have existed as commercial products for more than 60 years. It is can be naturally found in many fruit. Today, sorbitol is used in food, confectionary, oral care, pharmaceutical and industrial applications because of their unique physical and chemical properties which is as the starting material for the production of sorbose and ascorbic acid.

Sorbitol is suitable for a variety of products reduced in calories, sugar or fat and has been safely used for almost half a century. Sorbitol has relative sweetness of round sixty percent compared to sucrose with one-third fewer calories. In products, it not only fulfils a role as sweetener, but also as a humectants, texturizer and softener. It is also non-cariogenic and because of its benefits, it may be useful to people with diabetes (Kellen *et al*., 2007).

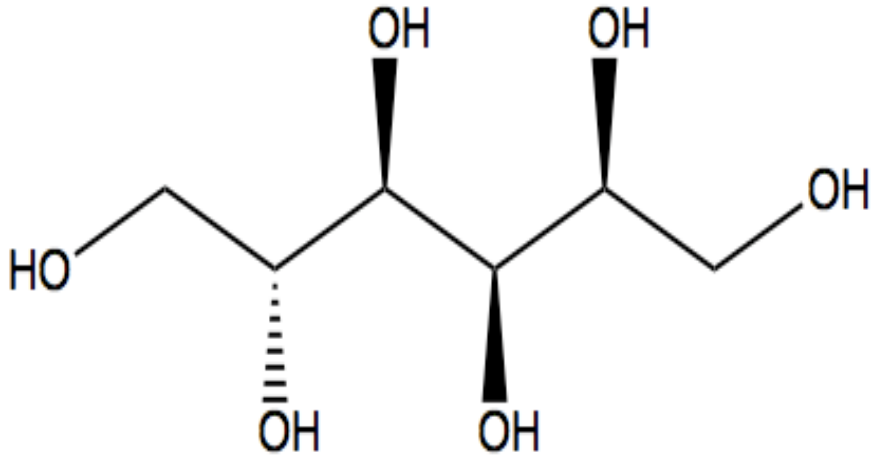

Figure 2.1: Chemical structure of sorbitol

2.2 Sorbitol Production by Fermentation

Several industrial processes have been described for the production of sorbitol as potential sorbitol produces, including fermentation process. Ladero states that production of sorbitol can achieved in bacteria. However, only few microorganisms have been described for the production of sorbitol. But, compared to the others, *Lactobacillus plantarum* bacteria can be utilized in fermentation process to achieve high level production of sorbitol from glucose (Ladero *et al.*, 2007).

2.2.1 pH of Medium

At the end of fermentation, pH was equal to 6.5 in order to maintain the pH growth of *Lactobacillus plantarum*. The pH is a key parameter which has to be taken into account when optimizing the separation process of fermentation broth by

microfiltration because of the broth pH, which is generally determined by the fermentation conditions that can affect the filtration performance (Milcent, 2001).

2.3 *Lactobacillus plantarum* Bacteria

Lactobacillus plantarum is a 0.3 µm in diameter and 8 µm long, rod shaped bacteria as shown in figure 2.2 (Ferrer, 2009). It is one of lactic acid, gram positive, nonsporulated and anaerobic bacteria which able to synthesis sorbitol, sugar alcohol from glucose by fermentation process where the growth and fermentation pH of the bacteria is at 6.5 (Patra *et al*., 1997).

Sabaitis (1976) states that the isoelectric point of *Lactobacillus plantarum* is about 3.75. The behavior of the lactic acid bacteria is depends on its surface properties. This is because of the cell surface of *Lactobacillus plantarum* that can adapt in responses to environmental change, like in low pH and ionic strength solution (Rodriguez *et al*., 2004).

Figure 2.2: *Lactobacillus plantarum* bacteria

2.3.1 Isoelectric Point of *Lactobacillus plantarum*

Isoelectric point is the pH at which a particular molecule or surface properties carries no net charge. The net charge on the molecule is affected by pH of their surrounding environment and can become more positive or negatively charged. Sabaitis states that the isoelectric point of *Lactobacillus plantarum* is at pH 3.75. Even though the *Lactobacillus plantarum* bacteria can adapt to environmental change, the different of pH can affect the surface charge of the bacteria if the pH is lower or higher that the isoelectric point of the bacteria (Manttari *et al*., 2006).

2.3.2 Cell Surface Properties

The electric charge is consequence of chemical composition of the surface layer protein conveys hydrophobicity to the *Lactobacillus plantarum* cell surface. This suggests that cell surfaces of *Lactobacillus plantarum* may adapt in response to environmental change like in pH or ionic strength. *Lactobacillus plantarum* is also a strong electron donor and weak electron acceptor. In other words, *Lactobacillus plantarum* bacteria have strong basic and weak acidic character (Pelletier *et al*., 1997).

2.4 Culture Medium of *Lactobacillus plantarum* Bacteria

The function of Man Rogossa and Sharpe (MRS) agar and broth is to provide a medium that would support the good growth of *Lactobacillus plantarum*. The ammonium citrate that contained in both the MRS agar and broth inhibits most microorganisms, but allows for the growth of *Lactobacillus plantarum*. The dipotassium phosphate and sodium acetate are buffer agents to maintain the pH of the agar and broth, tween 80 is an emulsier, manganese and magnesium sulfates are sources of ions and sulfate, peptone and meat extracts are nutrient sources for growth that contain nitrogen, vitamins, minerals and amino acids. In addition, dextrose is

the fermentable carbohydrate as carbon and energy source for the *Lactobacillus plantarum* bacteria (Briggs, 1960).

Table 2.1: Description of lactobacillus species

Species	Description	References
L. casei	Cell surface of Lactobacillus can adapt and response to pH and ionic strength	Rodriguez et al., 2005
L. casei	Lactobacillus strain slightly negatively charged at alkaline pH solution and positively charged with decreasing pH	Pelletier *et al*., 1997
L. plantarum	Suggested for use when bacteria need to adapt efficiently to environmental change	Koupion *et al*., 2007
L. plantarum	MRS broth culture maintained at pH 6	Todorov, 1999
L. plantarum	The cell harvested and washed with phosphate buffer pH 6.5	Rivas *et al*., 2008

2.5 Streaking Technique

Agar streak plates are an essential tool in culture process. The streaking technique which is used allows bacteria and fungi to grow on a solidified agar surface to produce discrete colonies. These colonies can be used to help identifying the organism, purify the strain free of contaminants, and produce a pure genetic clone. In order to obtain well isolated discrete colonies, the quadrant streak technique should be used because it allows sequential dilution of the original microbial broth or colonies on a plate (Thiel, 1999)

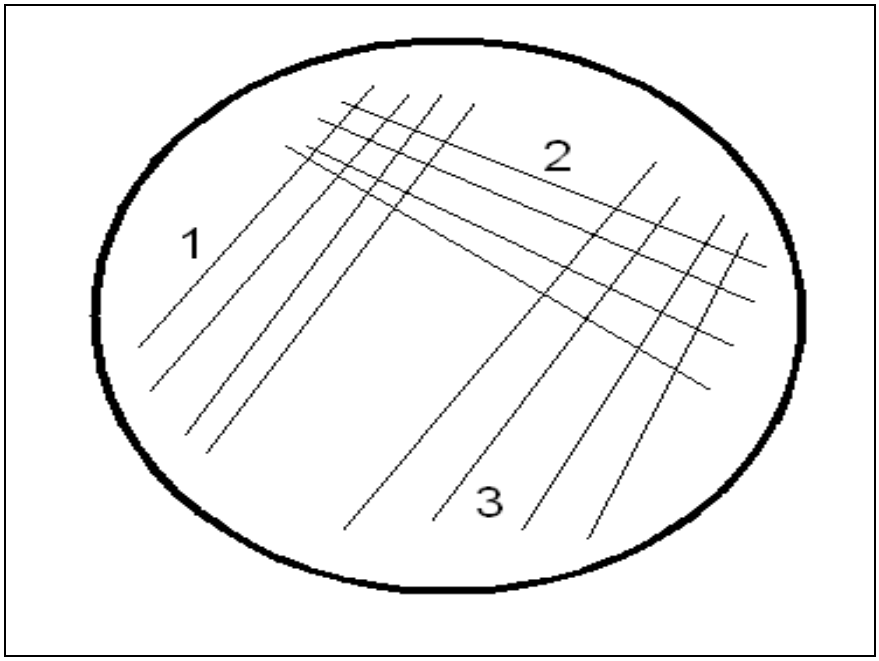

Figure 2.3: Streaking Technique

2.6 Membrane

A membrane can be described as a thin barrier between two bulk phases that permits transport of some components but retain others (Sarif, 2005). In order to allow the transport of material through a membrane, a driving force is necessary. The transport of material through a membrane could be driven by convection or by diffusion.

A membrane may be made from organic polymers or inorganic material such as glass, metals and ceramics or even liquids. The examples of polymeric or organic membranes including those made from polysulfone, cellulose, cellulose acetate, polyethersulfone and polyamide. But, the inorganic membranes can be made from ceramics, glass and stainless steel (Ghosh, 2006).

There are many ways to classify a membrane. From a structural point of view of membranes, basically membrane can be classified as symmetric or asymmetric and from a morphological point of view, membranes can be classified into two categories which are porous or dense (Sarif, 2005). Porous membrane has

tiny pores or pore networks and on the other hand, dense membrane do not have any pores (Ghosh, 2006).

2.7 Membrane Separation Process

Membrane separation involves partially separating a feed containing a mixture of two or more components by use of a semipermeable barrier, the membrane through which one or more of the species moves faster than another or other species. The transport of material through a membrane could be driven by convection or by diffusion or indeed by a combination of the two.

Convection based transport takes place due to transmembrane pressure and diffusion based transported utilizes the concentration difference of the transported species across the membrane as the driving force. Pressure driven membrane based bioseparation process can be classified into four types based on the size of the permeable species. They are microfiltration, ultrafiltration, nanofiltration and reverse osmosis process (Ghosh, 2006).

2.8 Microfiltration

Microfiltration (MF) is used for separation of fine particles or micron-sized particles such as bacteria from fluids. The separation limit of microfiltration falls within 0.02 to 10µm, which is placed coarse filtration and ultra filtration (Young *et al*., 1999). Microfiltration membranes are asymmetric, porous and retain particles by a purely sieving mechanism. In term of pressure, the transmembrane pressure ranges usually used for microfiltration ranges from 1 to 50 psig.

In addition, most microfiltration membranes capture particles by surface filtration which is the surface of the membrane. The applications of microfiltration in biotechnology include cell harvesting from bioreactors during fermentation

process. A microfiltration process can be operated either in a dead-end mode or cross-flow mode. But, for most applications, cross flow microfiltration is preferred (Ghosh, 2006).

2.8.1 Cross Flow Microfiltration

Cross flow microfiltration is a pressure driven membrane process in which the fluid to be filtered flows parallel to the membrane surface (Young *et al*., 1999). The configuration of cross flow microfiltration helps to reduce the formation of filter cake can allow a better permeate flux because cross flow microfiltration has a filtration surface which is continuously swept by flowing liquid. The shear of the flowing liquid along the tube wall minimizes the buildup of the solids on the microfiltration surface and hence, minimizes the fouling of membrane. Thus, cross flow microfiltration affords the possibility of nearly steady state operation.

The cross flow micro filtration modules contain multiple porous tubes, which have a nominal pore size of 0.2 microns. With this small pore size, large colloidal particles, and bacteria can be filtered from a fermentation process, but not molecular level substances (Moka *et al*., 2001)

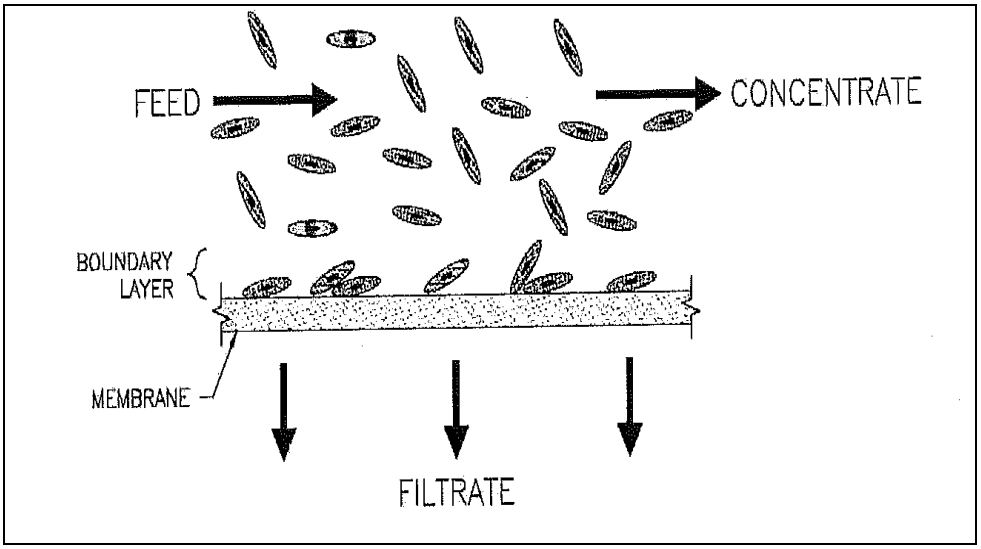

Figure 2.4: Cross flow mechanism